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Redescription of *Rhacophorus tuberculatus* (Anderson, 1871) and the validity of *Rhacophorus verrucopus* Huang, 1983

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Abstract

Rhacophorus tuberculatus and *Rhacophorus verrucopus* are two morphologically similar species described in 1871 and 1983 respectively. Their taxonomic distinctiveness has been questioned in the past. In the current study, we encountered frogs that we confer to *R. tuberculatus* based on morphological similarity to a syntype of this species. We redescribe the species based on a re-examination of a syntype, which is designated as a lectotype here, and additional specimens from Garo hills of Meghalaya. We also present molecular data, natural history notes, and report a range extension of this species. Molecular phylogenetic analysis based on the 16S rRNA fragment revealed minimal genetic divergences (0.20–1.74% uncorrected p-distance) between specimens identified as either *R. tuberculatus* or *R. verrucopus* from different locations. On the basis of molecular data and morphological characteristics, we conclude that *R. verrucopus* is a junior synonym of *R. tuberculatus*.

Key Words

Darwinian shortfall, Indo-Burma hotspot, range extension, Rhacophoridae, synonymy, systematics

Introduction

Frogs of the genus *Rhacophorus* Kuhl & Van Hasselt, 1822 occur across South and Southeast Asia and are represented by 44 extant species (Frost 2023). Despite many species of *Rhacophorus* being widespread, most of them are poorly studied, and information on their distribution is sparse (Ohler and Delorme 2005). *Rhacophorus tuberculatus* (Anderson, 1871) is one such poorly known species. It was described in 1871 from "Seebsaugor, Assam" (now Sivasagar 26.98515°N, 94.63878°E) and was subsequently reported from Arunachal Pradesh, Meghalaya, and West Bengal, India (Annandale 1912; Chanda 2002; Sen 2004; Das and Dutta 2007; Ahmed et al. 2009; Mathew and Sen 2010; Roy et al. 2018). Huang (1983) described *Rhacophorus verrucopus* from "Beibeng, Medo Xian" (Beibeng Township, Motuo County, Xizang Autonomous Region, China 29.23942°N, 95.17644°E), which closely resembles *R. tuberculatus* and this species was known only from its type locality and northern Myanmar (Fei 1999; Fei et al. 2009, 2010; Li et al. 2010; Fei et al. 2012; Liu et al. 2020; Fei 2020; Zug 2022). By studying the type and near-topotypical specimens of *R. verrucopus* and comparing them with the descriptions of *R. tuberculatus*, Che et al. (2020) considered that *R. verrucopus* and *R. tuberculatus* could be the same species.

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However, since Che et al. (2020) did not obtain any molecular data or examined the types of *R. tuberculatus* from India and only made comparisons of external morphology, Frost (2023) does not currently adopt their view and still treats *R. verrucopus* as a valid separate species.

We encountered a *Rhacophorus* species, the identity of which was difficult to determine, during our recent surveys between 2021 and 2022 in the West Garo hills of Meghalaya, India. Herein, we ascertain the identity of that *Rhacophorus* species as *R. tuberculatus* based on comparison with the syntypes of *R. tuberculatus* and provide a redescription of the species after more than 150 years since its original description, provide photographs of live individuals, morphological measurements and genetic divergence of *R. tuberculatus* with other congeners using 16S rRNA gene and discuss its relationship with *R. verrucopus*.

Materials and methods

Study area

We conducted surveys in Sasatgre village (25.5250°N, 90.3350°E, ca. 940 m) and Baladingre village (25.514213°N, 90.398204°E, ca. 835 m) of West Garo Hills district of Meghalaya between 2020 and 2022.

Voucher collection

Frogs were caught by hand, photographed first and euthanized using 20% Benzocaine following Torreilles et al. (2009). A small portion of the liver tissue was extracted by making a narrow slit on the ventral aspect of the specimens and stored in Molecular Biology Grade Ethanol (BP2818). Specimens were later fixed in 90% Ethanol for two hours and then transferred to 70% Ethanol for long-term storage as museum specimens. In total, seven individuals were collected for this study: six adult males collected from near Sasatgre village (25.5250°N, 90.3350°E, 940 m asl.) and an adult female from near Baladingre village (25.514213°N, 90.398204°E, 835 m asl.). The specimens used for morphological and molecular analyses were deposited at the herpetological collection facility at Sálim Ali Centre for Ornithology and Natural history (SACON).

DNA extraction and molecular analysis

Total genomic DNA was extracted from tissue samples of two specimens of *R. tuberculatus* (SACON VA 148 and VA 800) with a DNA extraction and purification kit, following the manufacturer's protocols. 16S rRNA gene was amplified using the primers 16sAR-L (5′-CGCCTGTTTATCAAAAACAT-3′) and 16sBR-H (5′-CCGGTCTGAACTCAGATCACGT 3′) respectively (Kocher et al. 1989). Amplifications were performed in an Applied Bio Systems Veriti 96 well thermal cycler: 20 µl reactions with 4 µl of 5× Phusion HF buffer, 0.4 µl of 10mM dNTP, 0.2 µl of Phusion DNA Polymerase, 0.1 µl each of forward and reverse primers, 2.0 µl of DNA template and 13.2 µl of nuclease free water with the following procedure: initial denaturation of DNA at 95 °C for 5 min, 35 cycles of: denaturation at 95 °C for 1 m, annealing at 55 °C for 1 min, extension at 72 °C for 1 m and at last, final extension at 72 °C for 10 min. The amplicon was checked by running it through an agarose gel electrophoresis for a clear band of the desired region in the amplified PCR product. The amplified PCR product was purified and sequenced commercially (National Centre for Biological Sciences, Bengaluru). Sequences were edited and manually adjusted using SeqMan in Lasergene 7.1 (DNASTAR Inc., Madison, WI, USA) and MEGA 11 (Tamura et al. 2021). Species of the genus *Zhangixalus* were selected as outgroups following Liu et al. (2022). Homologous and outgroup sequences were obtained from GenBank (Table 1). The technical computation methods for sequence alignment, genetic distance calculation, the best substitution model selection, Bayesian Inference (BI) and Maximum Likelihood (ML) phylogenetic analyses were the same as those in Liu et al. (2021).

Morphometric measurements

The following measurements were recorded to the nearest 0.02 mm from the specimens using an INSIZE dial caliper: snout–vent length (SVL, from the tip of the snout to the anterior margin of the cloaca), axilla–groin distance (AG, from the posterior margin of the forelimb at its insertion point on the body to the anterior margin of the hind limb at its insertion point on the body), head length (HL, from the posterior edge of the mandible to the tip of the snout), head width (HW, the maximum width of the head at the angle of the jaws), head depth (HD, the maximum depth of the head), body width (BW, the maximum width of the body at the trunk), eye diameter (ED, the greatest horizontal diameter of the orbit), eye– nostril distance (EN, from the anterior border of the orbit to the middle of the nostril), eye–snout distance (ES, from the anterior border of the orbit to the tip of the snout), upper eyelid width (UEW, the maximum width of the upper eyelid), interorbital distance (IO, distance between the margins upper eyelids), internarial distance (IN, distance between the nostrils), upper arm length (UAL, from the axilla to elbow), lower arm length (LAL, from the posterior margin of the elbow to the base of the outer metacarpal tubercle), palm length (PAL, from the posterior border of the outer metacarpal tubercle to tip of the 3rd finger), femur length (FEL, from the cloaca to the knee), tibia length (TBL, from knee to heel), foot length (FOL, from inner metatarsal tubercle to the top of the 4th toe). Webbing formulae follows Savage and Heyer (1997).

Geographic range estimation

Geographic range of the target species was calculated by plotting the known occurrences of the species on a

Table 1. List of specimens and GenBank accession numbers for all 16S rRNA sequences included in this study.

Taxon	Voucher No.	Locality	GenBank No.
Rhacophorus annamensis	VNMN 4090	Dak Nong, Nam Nung, Vietnam	LC010566
Rhacophorus baluensis	FM235958	Sabah, Malaysia	KC961089
Rhacophorus bengkuluensis	UTA A-62770	Lampung, Sumatra, Indonesia	KM212948
Rhacophorus bipunctatus	PUCZM/IX/SL360	Mizoram, India	MH087073
Rhacophorus borneensis	BORN:22410	Maliau Basin, Sabah, Malaysia	AB781693
Rhacophorus calcaneus	VNMN 4093	Dak Lac, Chu Yang Sin, Vietnam	LC010573
Rhacophorus catamitus	ENS 14726	Sumatra, Indonesia	KX398877
Rhacophorus exechopygus	VNMN 4107	Gia Lai, Kon Ka Kinh, Vietnam	LC010585
Rhacophorus helenae	AMS R 173230	Binh Thuan, Vietnam	JQ288087
Rhacophorus hoabinhensis	VNMN A.2016.16	Hoa Binh, Vietnam	LC331097
Rhacophorus indonesiensis	MZB: Amp:23619	Indonesia	AB983367
Rhacophorus kio	VNMN 4110	Gia Lai, Kon Ka Kinh, Vietnam	LC010589
Rhacophorus lateralis	SDB.2010.330	Karnataka, Bygoor, India	KC571277
Rhacophorus malabaricus	Rmal-In	Madikeri, India	AB530549
Rhacophorus margaritifer	ENS 16162	Java, Indonesia	KX398889
Rhacophorus modestus	ENS 16853	Sumatra, Indonesia	KX398904
Rhacophorus napoensis	GXNU YU000171	Napo, Guangxi, China	ON217796
Rhacophorus nigropalmatus	Rao081203	Malaysia	JX219438
Rhacophrus norhayatiae	NNRn	Endau Rompin, Johor, Malaysia	AB728191
Rhacophorus orlovi	VNMN 3067	Huong Son, Ha Tinh, Vietnam	LC010598
Rhacophorus pardalis	FMNH273243	Sarawak, Bintulu, Malaysia	JX219454
Rhacophorus poecilonotus	ENS 16480	Sumatra, Indonesia	KX398920
Rhacophorus pseudomalabaricus	SDB.2011.1010	Kerala, Kadalar, India	KC593855
Rhacophorus reinwardtii	Rao081205	Malaysia	JX219443
Rhacophorus rhodopus	SCUM 060692L	Mengyang, Yunnan, China	EU215531
Rhacophorus robertingeri	VNMN 4123	Gia Lai, Kon Ka Kinh, Vietnam	LC010613
Rhacophorus spelaeus	IEBR A.2011.1	Khammouan, Lao	LC331095
Rhacophorus translineatus	Rao6237	Motuo, Xizang, China	JX219449
Rhacophorus tuberculatus	KIZ014154	Motuo, Xizang, China	MW111522
Rhacophorus "verrucopus"	Rao6254	Motuo, Xizang, China	JX219436
	SEABRI2019120056	Htamanthi, Sagaing, Myanmar	MW275978
Rhacophorus tuberculatus	SACON VA – 148	Meghalaya, India	OR836578
	SACON VA – 800	Meghalaya, India	OR836579
Rhacophorus vampyrus	VNMN 4125	Hon Ba, Khanh Hoa, Vietnam	LC010616
Zhangixalus dennysi	SCUM 060401L	Shaoguan, Guangdong, China	EU215545
Zhangixalus dugritei	SCUM 051001L	Baoxing, Sichuan, China	EU215541

distribution map generated using ARCGIS 10.5. The area within the minimum convex hull was computed by connecting the outermost occurrence points to calculate the extent of occurrence as defined by the IUCN (2001).

Results

The suggested best substitution model for BI was GTR+F+I+G4 and for ML was TIM2+F+I+G4, both analyses showed an essentially consistent topology (Fig. 1). The sequences of the newly collected specimens from Meghalaya, India clustered with the sequences of *R. verrucopus* from Myanmar with strong supports by both BI and ML (0.97/99), and they together clustered with the sequences of *R. verrucopus* from China with strong supports by both BI and ML (1/100). The genetic divergence (uncorrected p-distance) between the sequences of the newly collected specimens and the sequences of *R. verrucopus* from China ranged from 1.50% to 1.74%, the genetic divergence (uncorrected p-distance) between the sequences of the newly collected specimens and the sequence of *R. verrucopus* from Myanmar ranged from 0.20% to 0.44% (Table 2).

Morphologically, the newly collected specimens (*R. tuberculatus*) from Meghalaya, India agree with the Syntype (ZSI 10154) and subsequent descriptions and figures by Annandale (1912) and Mathew and Sen (2010) of *Rhacophorus tuberculatus* in most aspects, especially in having a distinct tympanum, almost half as large as the eye; absence of vomerine teeth; presence of partial, sheath-like webbing on fingers and fully developed webbing on toes; pointed projection at tibio-tarsal articulation; well-developed and expanded discs on toe. Hence, we consider these newly collect-

Table 2. Genetic divergences (uncorrected p-distance in %) (%) between specimens identified as either *Rhacophorus tuberculatus* or *R. verrucopus* from different locations.

Species	Voucher	1	2	3	4
Rhacophorus tuberculatus	SACON VA – 148 India				
	SACON VA – 800 India	0.22			
Rhacophorus "verrucopus"	SEABRI2019120056 Myanmar	0.20	0.44		
	Rao6254 China	1.50	1.74	1.24	
	KIZ014154 China	1.54	1.74	1.28	0.00

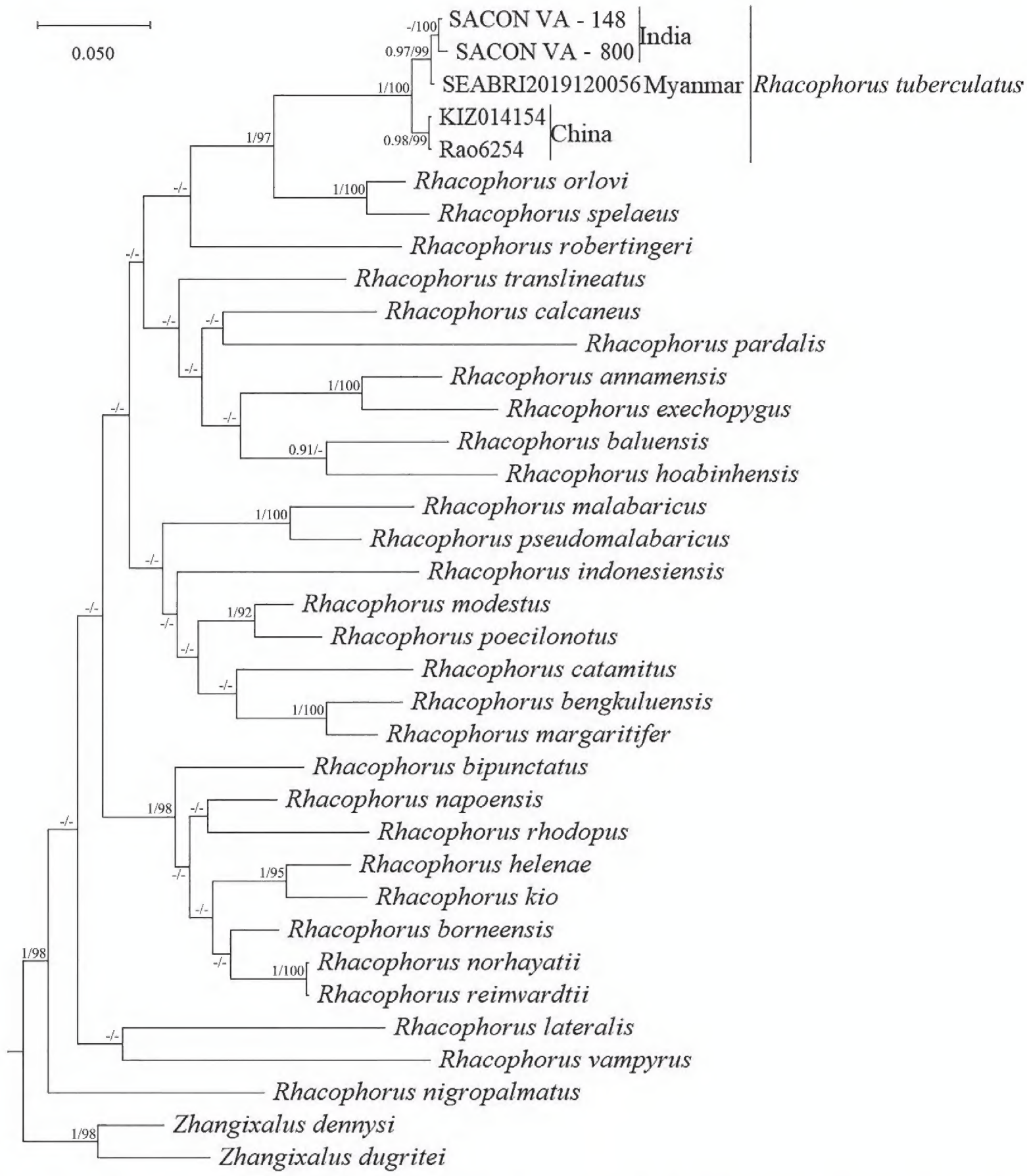

Figure 1. Bayesian inference tree of the genus *Rhacophorus* based on partial 16S rRNA fragments. Numbers before slashes indicate Bayesian posterior probabilities (≥ 0.90 remain) and numbers after slashes indicate ultrafast bootstrap support for ML analyses (≥ 90 remain).

ed specimens to belong to *R. tuberculatus*. In addition, there were also no obvious morphological differences between the newly collected specimens of *R. tuberculatus* and *R. verrucopus* from China and Myanmar (see Table 3).

Integrating the results of morphological data and also considering the shallow genetic divergence that is usually considered as intraspecific variation in the genus *Rhacophorus*, we second Che et al. (2020) in stating that *R. tuberculatus* and *R. verrucopus* are conspecific, and formally place *R. verrucopus* under the subjective synonymy of *R. tuberculatus*. Below, we give a formal synonymy list and provide the description of the specimen ZSI 10154 and designate it as the lectotype of *Rhacophorus tuberculatus*. Additionally, we also provide measurements of the newly collected material for comparison.

Systematics

Rhacophorus tuberculatus (Anderson, 1871)

Polypedates tuberculatus Anderson, 1871.
Rhacophorus tuberculata – Boulenger, 1882.
Rhacophorus (*Rhacophorus*) *tuberculatus* – Ahl, 1931.
Rhacophorus schlegelii tuberculatus – Wolf, 1936.
Rhacophorus verrucopus Huang, 1983, syn. nov.
Rhacophorus tuberculatus – Inger, 1985.
Rhacophorus (*Rhacophorus*) *verrucopus* – Dubois, 1987 «1986».

Redescription of *Rhacophorus tuberculatus* (Anderson, 1871). Specimens examined: ZSI 10154, lectotype by present designation, adult female, collected by Anderson from "Seebsaugor, Assam"; SACON VA – 143, 144, 145, 146, 147 and 148, adult males collected

Table 3. Morphometric measurements (in mm) of *R. tuberculatus* and *R. turpes* from the current study (*Designated as lectotype).

Species	*Rhacophorus tuberculatus* (Voucher no., sex and values)								*Rhacophorous turpes* (Voucher No., sex and values)	
Voucher no: Morphometric variables	*ZSI 10154	SACON VA 143	SACON VA 144	SACON VA 145	SACON VA 146	SACON VA 147	SACON VA 148	SACON VA 800	BMNH 1940.6.1.30	BMNH 1974.828-832
	Female	Male	Male	Male	Male	Male	Male	Female	Female	Female
SVL (mm)	39.10	30.32	29.38	30.88	28.16	27.02	30.76	34.02	37.42	35.80
AG	17.44	12.80	12.20	14.06	12.84	12.48	13.74	16.82	21.28	18.02
BW	16.92	7.52	5.82	7.28	6.76	6.10	8.44	7.44	14.22	11.46
HL	11.40	8.74	9.18	10.00	8.40	7.92	10.02	11.62	12.22	10.76
HW	11.10	9.38	9.00	9.34	8.92	8.86	9.40	12.00	9.74	9.78
HD	6.04	4.32	4.08	4.60	3.92	3.62	3.54	6.32	4.68	4.28
ED	4.42	4.28	4.10	3.70	3.70	3.84	4.18	4.90	3.54	2.98
EN	2.80	2.98	2.00	2.32	2.84	2.84	2.56	3.08	2.74	3.30
ES	4.42	3.78	3.64	4.32	4.52	4.22	4.32	5.62	4.90	5.43
TYD	1.82	1.68	1.80	2.08	1.20	1.72	1.70	2.38	2.44	2.76
ET	1.24	0.48	0.80	0.52	0.60	0.40	0.72	0.66	1.12	1.18
UEW	2.86	3.70	4.20	3.70	3.66	3.68	3.70	5.54	2.44	2.02
IO	4.64	3.12	3.70	2.82	2.98	2.72	3.02	4.14	4.28	4.12
IN	3.50	2.40	2.36	1.74	2.50	2.80	2.98	3.22	2.80	3.08
UAL	6.46	5.72	5.62	4.72	4.92	4.04	4.50	5.42	5.24	6.98
LAL	7.62	5.64	5.42	5.66	5.24	5.94	5.34	8.00	7.00	7.68
PAL	9.42	6.84	7.32	7.20	6.90	6.70	7.40	9.58	9.18	8.64
FEL	16.92	13.56	13.62	11.72	11.74	11.72	13.96	15.06	14.88	16.22
TBL	18.80	15.48	14.82	13.8	14.4	13.74	14.32	17.28	16.90	18.28
TAL	12.44	8.48	9.34	7.34	8.44	7.20	9.14	9.90	8.94	9.32
FOL	19.76	11.12	10.88	9.38	9.84	9.28	11.88	12.96	10.82	10.36

from near Sasatgre (25.5250°N, 90.3350°E, ca. 940 m asl.) by RSN between 13th – 26th May 2020 and SACON VA – 800 Adult female collected from near Baladingre (25.514213°N, 90.398204°E, ca. 835 m asl.) by RSN on 26th February 2022.

Diagnosis. *Rhacophorus tuberculatus* can be differentiated from all known congeners by the following suite of external morphological characters: small to medium adult size (mean SVL 29.4 mm; range 27.0–30.9 mm); distinct tympanum, almost half as large as the eye; absence of vomerine teeth; presence of a prominent calcar at tibio-tarsal articulation; presence of partial, sheath-like webbing on fingers and fully developed webbing on toes; well-developed and expanded discs on toes; and a dorsal colouration of uniform pale brown with mild traces of an irregular patch on the head and mossy greenish patches near the shoulders in some individuals.

Description of Lectotype (Fig. 2). Head flat, almost as long as wide (HL:HW 1.03); snout slightly pointed in dorsal view, rounded in lateral aspect, projecting slightly beyond margin of the lower jaw; canthus rostralis distinct, bluntly angular; nostrils much closer to tip of snout than eye; eyes large (ED:HL 0.39); tympanum distinct almost half as large as eye (TYD:ED 0.41); Supra-tympanic fold distinct, originating from the posterior of eye to the axilla; Upper eyelids wide, (UEW 2.86), narrower than the interorbital space (UEW:IO 0.62). Inter-orbital space broader than the inter-narial space (IO:IN 1.33). Upper arms short (UAL:SVL 0.17), shorter than the lower arms (UAL:LAL 0.85); palm length longer than the upper arms (UAL:PAL 0.69); pointed projection (calcar) at tibio-tarsal articulation; relative length of fingers I < II < IV < III, tips of all fingers with well-developed discs with distinct circum-marginal grooves. Fingers partially webbed. Relative length of toes I < II < III < V < IV; tips of toes with well-developed disks with distinct circum-marginal grooves; disks smaller than those of toes. Dorsal skin smooth; flanks wrinkled; underside of chin and chest smooth, abdomen and thigh coarsely granular; the granulation much denser around the cloacal region; outer margin of both limbs with low dermal ridges.

Colouration in preservative. Dorsal colouration uniformly pale brown with mild traces of an irregular bluish black patch on the head. Ventral aspect of body pale cream coloured (Fig. 2).

Colouration in life of frogs recorded from Meghalaya. Dorsum, overall pale to medium brown in colour with small scattered black dots and three or four dark blackish brown transverse bands across the thigh and tibial region, tarsus feet and webbing between toes orangeish-red in colour. Webbing in fingers translucently yellow. Ventral sides mild brownish white and groins, thighs and the rest of the legs brownish yellow to dark red in colour. Some individuals with irregular florescent green patches on the head and mid body region (Fig. 4).

Natural history. The specimens of *R. tuberculatus* from Meghalaya examined during this study were collected from two different locations within West Garo Hills. A small shallow stream running parallel to the eastern boundary of the Sasatgre community reserve, the stream was bounded on both side by cardamom and banana plantations. The frogs were encountered at 1900 – 2300 hrs in the month of May, found perched on leaves of yam and cardamom plants, one to two meters above ground level. The other location was a similar habitat from another cardamom plantation near a forest patch near Baladingre village.

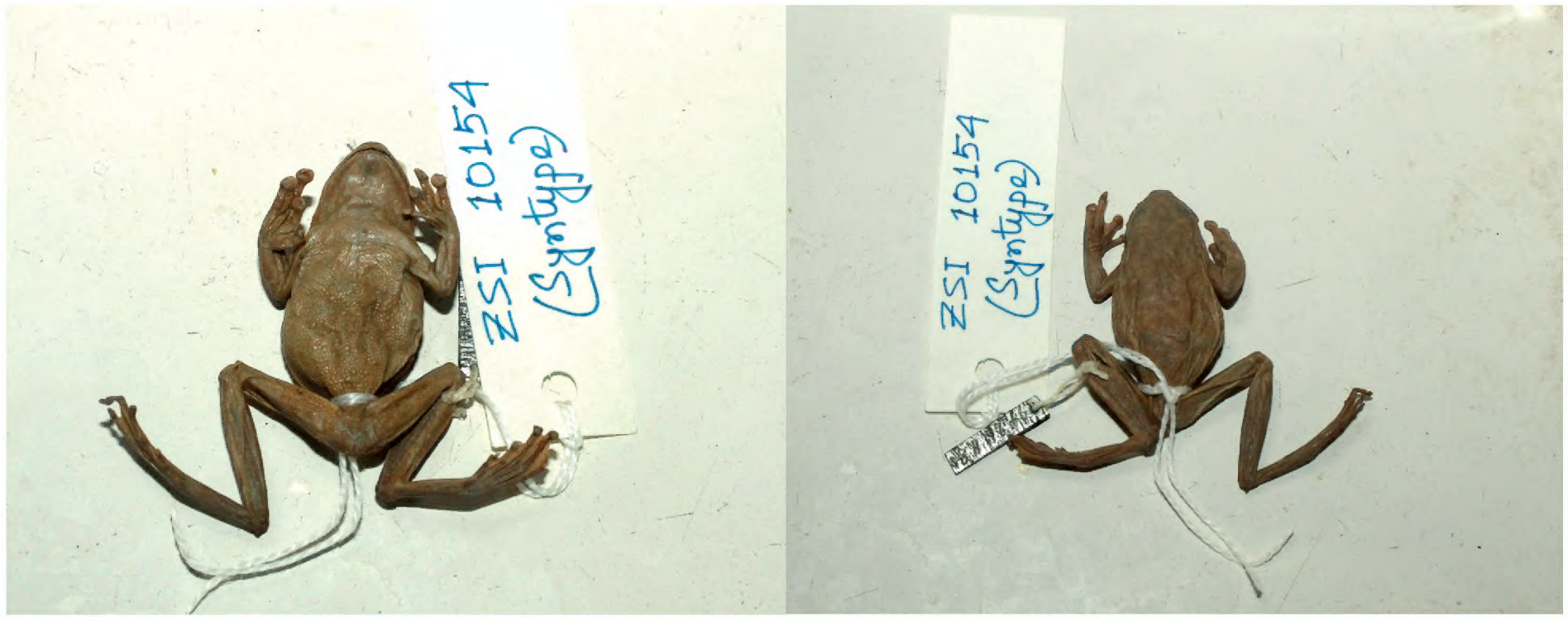

Figure 2. The ventral and dorsal view of the lectotype of *Rhacophorus tuberculatus* ZSI 10154.

Figure 3. The ventral and dorsal view of a syntype of *Rhacophorus turpes* BMNH 1940.6.1.30.

Figure 4. *Rhacophorous tuberculatus* in life from Garo hills, Meghalaya.

Table 4. Morphological (in mm) comparisons between our newly collected specimens of *Rhacophorus tuberculatus* from India and *R. verrucopus* from China and Myanmar. Data for *R. verrucopus* from China were obtained from Huang (1983) and Che et al. (2020), and data for *R. verrucopus* from Myanmar were from Liu et al. (2020) (" – " data unavailable).

Morphological variables	*R. tuberculatus*		*R. verrucopus*		*R. verrucopus*
	India		China		Myanmar
	Mean (Range) Male, n=6	Female, n=1	Mean (Range) Male, n=7	Female, n=1	Female, n=1
SVL	29.4 (27.0–30.9)	34.00	37.9 (36.0–40.6)	41.60	52.00
HL	9.0 (7.9–10.0)	11.60	12.5 (12.0–13.0)	12.20	17.60
HW	9.2 (8.9–9.4)	12.00	11.4 (10.9–12.3)	11.60	15.70
ED	4.0 (3.7–4.3)	4.90	4.3 (3.9–4.9)	5.20	5.40
ES	4.1 (3.6–4.5)	5.60	5.7 (5.1–6.7)	5.90	7.70
TYD	1.7 (1.2–2.1)	2.40	2.2 (2.0–2.4)	2.70	3.10
UEW	3.8 (3.7–4.2)	5.50	2.9 (2.5–3.6)	3.40	–
IO	3.1 (2.7–3.7)	4.10	4.3 (3.8–5.0)	3.60	–
IN	2.5 (1.7–3.0)	3.20	3.5 (3.2–4.1)	3.10	4.30
LAL+ PAL	12.6 (12.1–12.9)	17.60	16.5 (16.0–17.0)	18.80	17.80
PAL	7.1 (6.7–7.4)	9.60	10.6 (10.1–11.1)	11.70	–
FEL	12.7 (11.7–14.0)	15.10	17.3 (16.3–17.9)	20.50	22.90
TBL	14.4 (13.7–15.5)	17.30	17.9 (17.3–18.3)	21.20	24.20
FOL	10.4 (9.3–11.9)	13.00	15.3 (14.6–16.2)	19.00	20.40
HL/SVL	0.31 (0.29–0.33)	0.34	0.33	0.29	0.34
HL/HW	0.99 (0.89–1.07)	0.97	1.10	1.05	1.12
ED/HL	0.44 (0.37–0.49)	0.42	0.35	0.43	0.31
TYD/HL	0.19 (0.14–0.22)	0.21	0.17	0.22	0.18
FEL/SVL	0.43 (0.38–0.46)	0.44	0.46	0.49	0.44
TBL/SVL	0.49 (0.45–0.51)	0.51	0.47	0.51	0.47
TBL/FEL	1.14 (1.03–1.23)	1.15	1.04	1.03	1.06

Discussion

"Darwinian shortfall" is a major challenge faced by conservationists today, the lack of availability of molecular data for several extant species is a common phenomenon across taxa, leading to a situation where phylogenetic information is absent for most organisms, thus inhibiting a robust understanding of phylogenetic relationships within a particular group (Diniz-Filho et al. 2013). *Rhacophorus tuberculatus* is one such species which was described by Anderson (1871), based on three adult specimens. The original description was brief and lacked photographs or diagrams since it was from more than 150 years ago. Although several subsequent studies such as Ahmed et al. (2009) and Roy et al. (2018) reported photographic records of this species, there had been no studies involving a detailed taxonomic assessment of the species to date. As a result, the species had been overlooked in several studies, including the description of *Rhacophorus verrucopus* by Huang (1983). The validity of this species has been doubted by Che et al. (2020) who suggested that *R. verrucopus* could be a junior synonym of *R. tuberculatus* but due to the lack of molecular evidence this was not accepted. In the current study, we provide genetic divergence between *R. tuberculatus* and other congeners, including '*R. verrucopus*' using 16s rRNA gene, thus filling an important knowledge gap. Further examination of *R. verrucopus* from China and Myanmar and *R. tuberculatus* from India based on re-examination of types and also freshly collected specimens used in this study revealed that there is an extensive overlap in morphology, and shallow molecular divergence (of a level that usually qualifies to be considered an intraspecific variation in the genus) between *R. verrucopus* and *R. tuberculatus*. Based on these lines of evidence we endorse the conclusions of Che et al. (2020) and formally place *R. verrucopus* under the junior synonymy of *R. tuberculatus*. Examination of the types (BMNH 1940.6.1.30 and BMNH 1974.828-832) of *Rhacophorus turpes* described from Kachin region of Northern Myanmar (26.24972°N, 97.23878°E) by Smith (1940) revealed that these specimens also closely match the morphological characters of *R. tuberculatus* except for a slightly truncated snout, a relatively less prominent tibio-tarsal projection, and fewer granulations on the ventral surface (Fig. 3). However, further studies utilizing molecular approaches based on fresh material are necessary to assess the taxonomic status of this species.

The current study also addresses the re-assessment of the threat status of *R. tuberculatus* as per IUCN Red List criteria of this Data Deficient species. *Rhacophorus tuberculatus* was known with certainty only from its type locality until now. However, in this study, we were able to resolve the taxonomic confusion with this species thus mapping its actual distribution range for the first time. The new records of this species from West Garo hills of Meghalaya mark the westernmost limit of the distribution of the species (Fig. 5). The up-listing or down-listing of species from one threat category to another of the IUCN Red List requires an assessment against all the five criteria (A–E, with 11 sub-criteria) but only one criterion needs to be fulfilled for designation of threat categories (IUCN 2001). In the case of *R. tuberculatus*, the information on geographic distribution seems the most accurate and reliable among all other criteria and hence, used for a conservative estimate of the extent of occurrence (Criteria B, B1). The current Extent Of Occurrence for this species estimated based on all known localities is about 1,07,600 km^2 and hence,

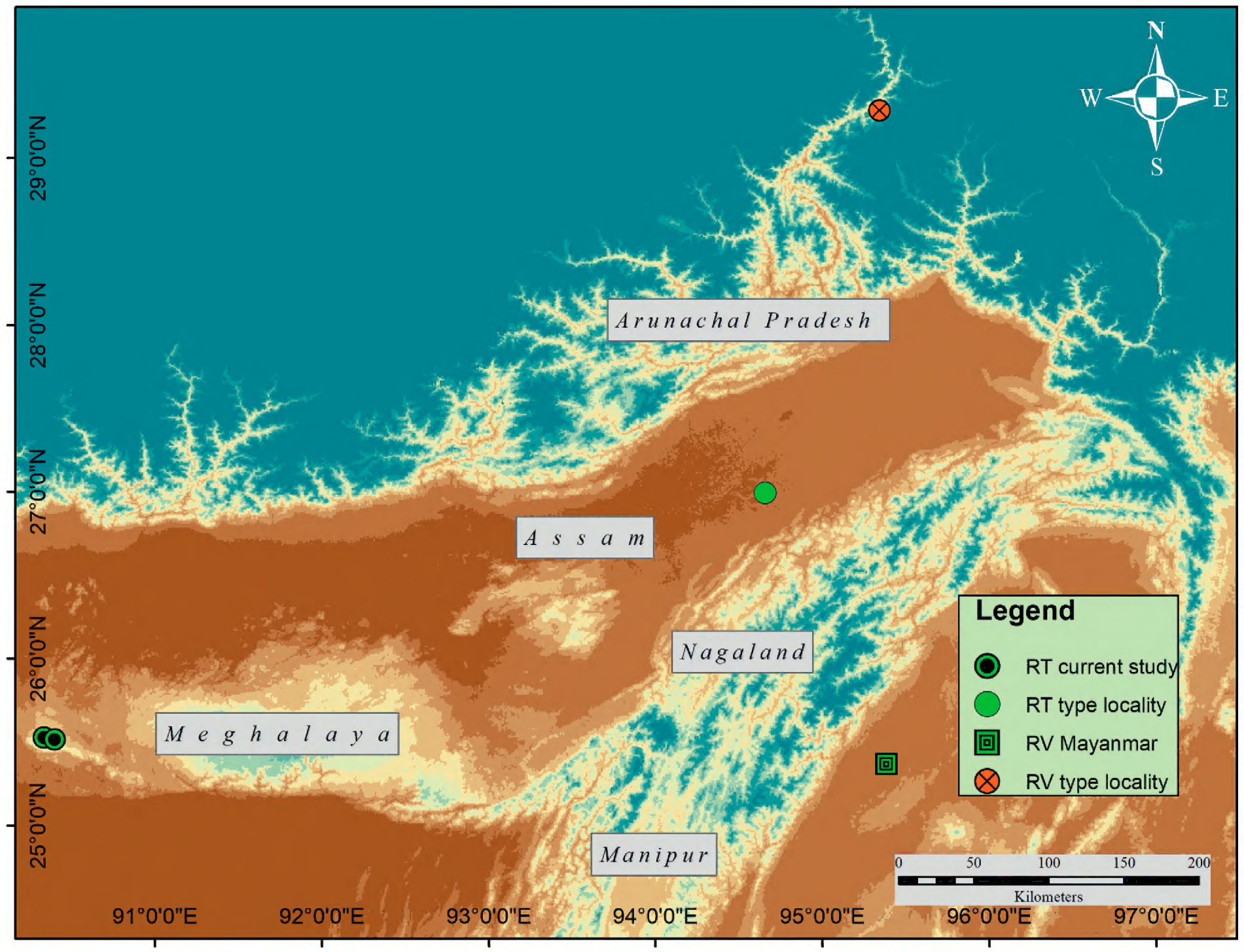

Figure 5. Updated distribution map of *R. tuberculatus* including records from Meghalaya (Current study) in green and black circle, from Myanmar in green and black square, type locality of "*R. verrucopus*" in red circle and type locality of *R. tuberculatus* in green circle.

with all the updated information presented here, we recommend transferring the species from Data Deficient to Least Concern.

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